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(54) A LUBRICATING COMPOSITION FOR MUCOSA OR EPIDERMIS

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the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention concerns components for lubricating compositions and, in particular, it relates to a lubricating composition useful for facilitating parturition in animals.

A fetus of an animal, for example, a cow, is enveloped doubly with amnion and chorioid. Inside the amnion, there is amniotic fluid, and inside the chorioid there is urine, At parturition, an embryonal sac covering the fetus is forced out with labour-pain, and a rupture of the chorioid happens first, thereby discharging the urine (the first rupture), and subsequently a rupture of the amnion occurs thereby discharging the amniotic fluid (the second rupture). The amniotic fluid is a specific high polymer solution which has a fluidity resembling that of albumen and is characterised by high mucous and thread-like properties. By thread-like properties of a liquid we mean a marked tendency for the liquid to be drawn out in a thread-like form when an article is withdrawn from the liquid. It covers the fetus at parturition and serves to lubricate the birth canal, thereby facilitating delivery of the fetus.

The amount of amniotic fluid is sufficient in the case of normal parturition, but if, for example, the labour time is long the amount of amniotic fluid becomes insufficient and thus a difficult delivery results. Furthermore, as a result of recent high domestication (an increase of physique and insufficient exercise) of cattle, swine, and other domestic animals, the time required for parturition is likely to be long in these domestic animals and this may result in undesirable effects such as damage to the birth canal and the fetus.

We have investigated the lubricating property of the amniotic fluid with a view to finding a substitute that will serve to lubricate a birth canal and thereby facilitate delivery of a fetus. We found that alginic acid salts by themselves were inadequate as the lubricating agent in lubricating compositions for the above purpose. Surprisingly, however, we found that a mixture of an alginic acid salt and fucoidin is very satisfactory as the lubricating agent in such compositions. Furthermore, in accordance with the invention, we have devised a convenient way of obtaining compositions comprising an alginic acid salt and fucoidin and, in particular, have obtained such compositions in particulate form. A lubricating composition according to the present invention comprises a solution of an alginic acid salt and fucoidin in water: these compositions have marked mucous and thread-like properties.

Several methods are known for the preparation of alginic acid or its alkali salts from algae of a *Phaeophyceae* species, for instance, the "acid-neutralizing method" illustrated in the flow sheet of Figure 1 of the accompanying drawings and the "calcium method" illustrated in the flow sheet of Figure 2. No method has, however, been known for preparing an alginic acid salt and fucoidin coincidentally from the *Phaeophyceae* nor have mixtures of these substances been used as a lubricant for living bodies.

We noted that both fucoidin and alginic acid salts are present in the *Phaeophyceae* such as sea tangle and ecklonia and by studying and investigating their properties we devised a method for extracting them coincidentally from the *Phaeophyceae*.

Fucoidin is a substance contained in cell-membranes of the *Phaeophyceae* and is a metal salt of polyglycosulphate composed mainly of fucose or grape sugar. The chemi-

cal properties of fucoidin are different from those of alginic acid or its salts in the following respects:

1. The alginic acid salts are converted to a water insoluble free acid in the presence of acids, but the free acid is readily converted to the original salts by neutralization.

Fucoidin is decomposed by acids. If neutralization of the decomposed product is effected it is found that the viscosity and other properties of fucoidin cannot be restored.

2. In the presence of calcium salts, alginic acid is converted to its water insoluble calcium salt and is precipitated.

Fucoidin is not influenced at all by the presence of calcium salts, and does not form any precipitate with them.

3. In the presence of alkalis, alginic acid is converted to a water-soluble alkali salt of alginic acid.

Fucoidin is not affected by alkalis.

Because of the differences described above, fucoidin is not extracted at all by the conventional methods (see Figures 1 and 2) for preparing alginic acid or its alkali salt, since these methods result in the decomposition of the fucoidin.

We have now confirmed and discovered the following facts:

1. Fucoidin reacts with aluminium or iron. Alginic acid and its alkali salts also react with these trivalent metals to form insoluble salts.

2. When an aluminium or iron salt of fucoidin or alginic acid is washed with an excess of caustic alkali, dissolved in a hydrophilic organic solvent mixed with a suitable amount of water, such as 70% ethanol, 70% methanol and 60% isopropanol, the aluminium or iron is eluted out due to the formation of a water-soluble complex salt, whereas fucoidin or alginic acid remains as a precipitate.

3. The product obtained by replacing calcium in fucoidin by the alkalis is water-soluble, and the solution has a thread-like property.

According to the present invention a process for preparing a composition comprising fucoidin and an alginic acid salt comprises treating, at a temperature of 30 to 80°C algae of *Phaeophyceae* species with an aqueous solution comprising a hydroxide, carbonate or phosphate of a monovalent metal or of magnesium, filtering the solution so obtained, adding an aluminium or iron salt to the filtrate at a pH of 6.5 to 9.0, immersing the resultant precipitate in a hydrophilic organic solvent, removing the aluminium or iron from the precipitate by adding a base selected from caustic soda, caustic potash and ammonia, water being present with the organic solvent and/or with the base, the aluminium or iron water-soluble complex salt thus formed being eluted into the liquid phase, and recovering and drying the precipitate.

The essential steps of the process and certain optional steps are indicated in Figure 3 of the accompanying drawings and are further described in the following.

1. A *Phaeophyceae* species seaweed is treated as described above with an aqueous solution of a hydroxide, carbonate or phosphate: this is the "alkali-dissolution" step of Figure 3. In seaweed alginic acid is usually present in the form of a calcium salt. Accordingly, it is preferred to use as reagent, a compound capable of readily forming an insoluble calcium salt by double decomposition, for example alkali carbonates and phosphates, rather than caustic alkalis. Preferably the reagent is capable of softening and dissolving the tissue of the seaweed and we have found that sodium pyrophosphate and/or sodium carbonate are particularly suitable for this purpose.

The first step is generally carried out for 2 to 4 hours and the preferred temperature is 50 to 65 degrees C.

The product of the first step e.g. a paste-like liquor is normally diluted until, for example, the alginic acid concentration is 0.3 to 0.5% by weight, before the filtration is carried out. The filtration can be carried out by conventional means. Since, however, the thread-like properties of liquid, fucoidin-containing compositions are easily destroyed by mechanical shearing forces, high pressure filtration is to be avoided. Also, in transporting the liquid a pump should not be used which exerts a violent shearing force.

The step of adding the aluminium or ferric salt (the trivalent metal salt of Figure 3) results in precipitation of the aluminium or iron salts of fucoidin and alginic acid. The salt used may be, for example, a hydrochloride, sulphate, acetate or an alum. The amount used should be sufficient to precipitate all the fucoidin and alginic acid as their salts. The pH is preferably 7.0 to 7.8. If the pH is too acid, fucoidin is destroyed whilst it is too alkaline, the precipitation becomes ineffective.

The precipitate obtained by use of the aluminium or iron salt is treated in a hydrophilic organic solvent with the base: this is the "alkali-washing" step of Figure 3. Preferred hydrophilic organic solvents are methanol, ethanol and iso-propanol. As the function of this step is to remove the aluminium or iron from the precipitate by formation of a water-soluble aluminium or iron complex salt with the base, the liquid present must contain water. This is achieved by initially using a hydrophilic organic solvent containing water and/or adding water with the base. In fact the organic solvent is

normally used as a mixture containing water and it is also convenient for the base to be added in the form of an aqueous solution.

The water-containing liquid elutes the complex salt from the precipitate and this salt can be removed from the remaining precipitate by washing. Accordingly, the relative proportions of the solvent and water are desirably such that all the complex salt is readily eluted out and removable by washing whilst fucoidin and alginic acid salts are not eluted from the precipitate at all. We have found, experimentally, that the preferred solvent concentration is 50 to 85% in the case of methanol, 45 to 80% in the case of ethanol and 45—75% in the case of iso-propanol. The amount of base used is preferably 0.5 to 2% by weight based on the organic solvent and water.

The precipitate remaining after the above step may be washed two or three times with a hydrophilic organic solvent in order to remove residual base. This step may, however, be omitted or replaced by neutralizing the residual base with a weak acid.

The precipitate is eventually recovered and dried to give a solid product.

When the solid product, e.g. in particulate form, obtained by the above mentioned steps is dissolved in water, it gives a solution that has excellent lubricating properties and exhibits a thread-like property. Preferably the solid product contains at least 5% by weight of fucoidin and preferably it contains at least 5% by weight of alginic acid salt. The solution can be used very conveniently as an agent for lubricating the birth canal at the time of parturition in cattle, swine and other animals. Furthermore it has been confirmed that attainment of similar effects in human bodies is also certainly to be expected. The solutions preferably have a total concentration of fucoidin and alginic acid salt of at least 1% by weight.

In one of our clinical experiments made on pregnant women, it was found that the delivery of a fetus could be greatly facilitated. In particular, damage to birth canal was prevented, the pressure imposed on the head of the neonate being reduced and the mother being released from labour pains, by injecting a 1% solution of the powdery product obtained in Example 1 given below in sterile water into the vagina just before parturition by means of a sterile injector. Furthermore no residual side effects were observed. Accordingly, it has been confirmed that the composition can be effectively applied both to animals and to humans. As both fucoidin and alkali salts of alginic acid are natural substances, there is no problem of side effects on animals that might result from use of synthetic substances.

The composition may be used as a lubricant for mucosa or epidermis in the various animals. For instance, when a veterinary surgeon treats a big animal by inserting his arm into a rectum or vagina, the insertion can be much facilitated by applying the composition around the arm.

The composition of this invention may optionally contain carboxymethyl cellulose (C.M.C.), gum arabic, sodium polyacrylate, potassium sodium polyphosphate or polyethylene oxide. C.M.C. and gum arabic like fucoidin and alkali salts of alginic acid, will dissolve to give viscous solutions of low structural viscosity having liquid characteristics close to Newtonian flow. The other substances just mentioned have, like fucoidin, thread-like properties in solution.

In order to heighten the solubility of the composition it is preferred at the time of application to blend it with glucose or lactose, or to add a surfactant or a coating agent to the composition.

It is also possible to mix an antiseptic or a curing medicine into the composition.

The invention is illustrated by the following examples.

EXAMPLE 1

10 kg of ecklonia collected at Shirahama, Chiba-Prefecture, Japan was cut into squares of about 1 cm, and 1 kg of sodium tertiary phosphate, 1 kg of sodium pyrophosphate, 0.5 kg of sodium carbonate and 50 liters of water were added thereto. Then, the blend was heated at 50°C and agitated for 2 hours. Thus there was formed a paste-like liquor. The liquor was diluted and filtered, and 20 liters of a 10% solution of aluminium chloride was gradually added to the filtrate. The resultant precipitate was subjected to centrifugal separation to remove water therefrom. The precipitate was immersed in 20 liters of 75% methanol, followed by addition of 2 liters of 30% caustic soda. The agitation was conducted for 1 hour and the precipitate was recovered by filtration. This alkali-washing treatment was repeated twice. Then, the precipitate was washed with 75% methanol free of caustic soda.

The resultant precipitate was dried to obtain 1.6 kg of a yellowish brown massive product, which was determined to have the following composition:

alginic acid salt	61.8%
fucoidin	22.2%
protein admixtures	1.0%
water	15.0%

The above product was pulverized to a powdery product.

The so obtained powdery product was easily soluble in water, and its aqueous

solution had a very marked thread-like property.

In order to test the properties of the product, a 1.5% aqueous solution of the product was prepared, which was a mucous liquid having a highly thread-like property and exhibiting an excellent lubricating property. Good lubricating effects were observed when the above aqueous solution was injected into a birth canal of a cow at the time of parturition.

Also good results were obtained when a concentrated solution of the above product having a concentration of 5—10% was applied directly to a birth canal or fetus at the time of parturition in a cow.

EXAMPLE 2

10 kg of arthrothamnus collected at Nemuro, Hokkaido, Japan, was cut into squares of about 10 cm, and 1.5 kg of sodium pyrophosphate, 1.5 kg of sodium tertiary phosphate and 50 liters of water were added thereto. The blend was heated at 60°C and agitated for 1 hour to effect dissolution. The solution was diluted 10 times and filtered. 22 liters of a 10% solution of aluminium sulphate was gradually added to the filtrate, and the resultant precipitate was subjected to the centrifugal separation. Then, the precipitate was immersed in 20 liters of 65% isopropanol, followed by addition of 2 liters of 30% caustic soda. The agitation was conducted for 1 hour, and the precipitate was recovered by filtration. This alkali-washing was repeated twice. Then, the precipitate was washed in 20 liters of 75% methanol.

The resultant precipitate was dried to obtain 1.7 g of yellowish brown massive product, which was determined to have the following composition:

alginic acid salt	62.3%
fucoidin	22.0%
protein admixtures	1.1%
water	14.6%

The massive product was pulverized to obtain a powdery product.

The product was dissolved in water to prepare an aqueous solution having a concentration of 1.5%. This aqueous solution was very mucous and excellent in lubricating property. When the solution was used at the time of parturition of a cow, excellent lubricating effects were obtained.

EXAMPLE 3

10 kg of ecklonia maxima produced in South Africa was pulverized to about 20 mesh (Tyler scale), and was incorporated with 1.0 kg of sodium carbonate, 1.5 kg of sodium tertiary phosphate, 1.5 kg of sodium pyrophosphate and 50 liters of warm water maintained at 30°C. The blend was allowed to stand still over-night, and heated at 80°C for 2 hours under agitation. The resulting solution was diluted 15 times and filtered. 25 liters of a 10% solution of ferric chloride was gradually added to the filtrate. The resultant precipitate was recovered by the centrifugal separation, and then immersed in 25 liters of 75% methanol, followed by addition of 2.5 liters of 30% caustic soda. The agitation was carried out for 1 hour and the precipitate was recovered by filtration. This alkali-washing treatment was repeated twice. Then, the precipitate was washed with 20 liters of 75% methanol, and dried to obtain 1.9 kg of a faintly yellowish brown massive product, which was determined to have the following composition:

alginic acid salt	65.4%
fucoidin	18.1%
protein admixtures	1.9%
water	13.6%

The masive product was pulverized to form a powdery product.

When the powdery product was used at the time of parturition of a cow in the same manner as described in Example 1, very excellent lubricating effects were obtained.

EXAMPLE 4

10 kg of ecklonia collected at Miura Peninsula, Kanagawa-Prefecture, Japan, were cut into squares of 2 cm, and 1 kg of sodium tertiary phosphate, 1 kg of sodium carbonate and 70 liters of water were added thereto. The blend was heated at 50°C for 2 hours under agitation. The resulting paste-like liquor was diluted and filtered. Then, 25 liters of a 10% solution of potassium alum ($KAL(SO_4)_2.12H_2O$) was gradually added to the filtrate. The resulting precipitate was treated in the same manner as in Example 1 to obtain 1.8 kg of a massive product which was determined to have the following composition:

alginic acid salt	56.0%
fucoidin	23.4%
protein admixtures	4.6%
water	16.3%

EXAMPLE 5

A filtrate obtained by repeating the first stage of the treatment of Example 2 was gradually incorporated with 20 liters of a 10% solution of iron alum ($Fe(NH_4)(SO_4)_2.12H_2O$), and the resultant precipitate was treated in the same manner as in Example 2 to obtain 1.65 kg of a massive product.

EXAMPLE 6

10 kg of ecklonia produced at Onjuku,

Chiba-Prefecture, Japan, was cut into squares of about 1 cm and blended with 1 kg of sodium tertiary phosphate, 1 kg of sodium pyrophosphate, 0.5 kg of sodium carbonate and 50 liters of water. The blend was heated at 60°C for 2 hours under agitation to obtain a paste-like liquor. The liquor was diluted and filtered. 20 liters of a 10% solution of ferric acetate was gradually added to the filtrate, and the resulting precipitate was subjected to centrifugal separation to remove water therefrom. The recovered precipitate was immersed in 20 liters of 75% methanol, followed by addition of 2 liters of 30% caustic soda. The agitation was conducted for 1 hour and the precipitate was recovered by filtration. This alkali-washing treatment was repeated twice. The resultant precipitate was washed with 75% methanol free of caustic soda and dried to obtain 1.5 kg of a yellowish brown massive product, which was determined to have the following composition:

alginic acid salt	58.9%
fucoidin	24.0%
protein admixtures	0.8%
water	16.3%

The above massive product was pulverized into a powdery product. The product was formed into a 1.5% aqueous solution, which was a mucous liquor having a high lubricating property. When the solution was used at the time of parturition of a cow in the same manner as in Example 1, excellent lubricating effects were obtained.

WHAT WE CLAIM:—

1. A particulate composition comprising fucoidin and an alginic acid salt.

2. A composition according to claim 1 containing at least 5% by weight of fucoidin.

3. A composition according to either preceding claim containing at least 5% by weight of alginic acid salt.

4. A composition according to claim 1 substantially as hereinbefore described with reference to any of the Examples.

5. A liquid composition comprising a solution of fucoidin and alginic acid salt in water.

6. A composition according to claim 5 in which the total concentration of fucoidin and alginic acid salt is at least 1% by weight.

7. A composition according to claim 5 substantially as hereinbefore described with reference to any of Examples 1 to 3 and 7.

8. A process for preparing a composition comprising fucoidin and an alginic acid salt comprising treating at a temperature of 30 to 80°C algae of a *Phaeophyceae* species with an aqueous solution comprising a hydroxide, carbonate or phosphate of a monovalent metal or of magnesium, filtering the solution so obtained, adding an aluminium or iron salt to the filtrate at a pH of 6.5 to 9.0 immersing the resultant precipitate in a hydrophilic organic solvent, removing the aluminium or iron from the precipitate by adding caustic soda, caustic potash or ammonia, water being present in the organic solvent and/or the base, the aluminium or iron water soluble complex salt thus formed being eluted into the liquid phase, and recovering and drying the precipitate.

9. A process according to claim 8 in which the hydrophilic organic solvent is a water-soluble alkanol.

10. A process according to claim 8 substantially as hereinbefore described with reference to any of the Examples.

11. A composition comprising fucoidin and an alginic acid salt prepared by a process according to any of claims 8 to 10.

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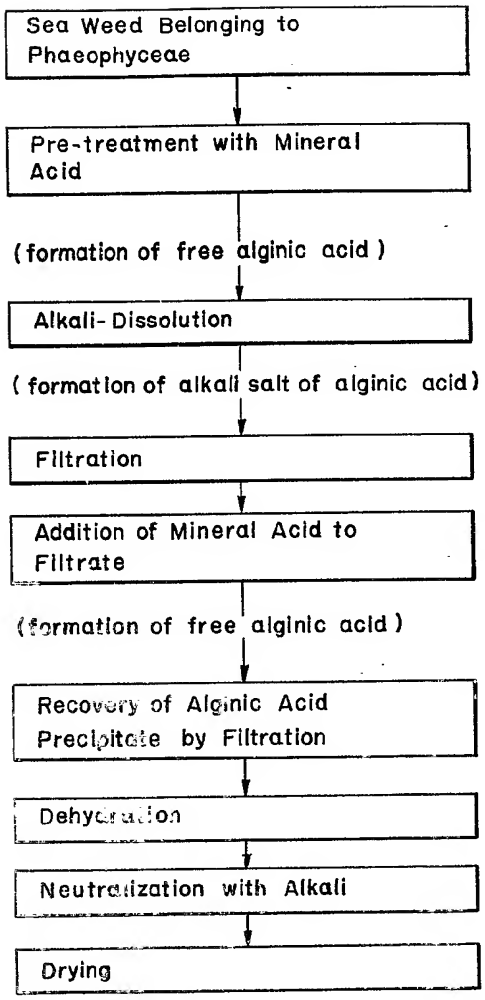
Sea Weed Belonging to Phaeophyceae
Pre-treatment with Mineral Acid
(formation of free alginic acid)
Alkali-Dissolution
(formation of alkali salt of alginic acid)
Filtration
Addition of Mineral Acid to Filtrate
(formation of free alginic acid)
Recovery of Alginic Acid Precipitate by Filtration
Dehydration
Neutralization with Alkali
Drying

FIG. 1

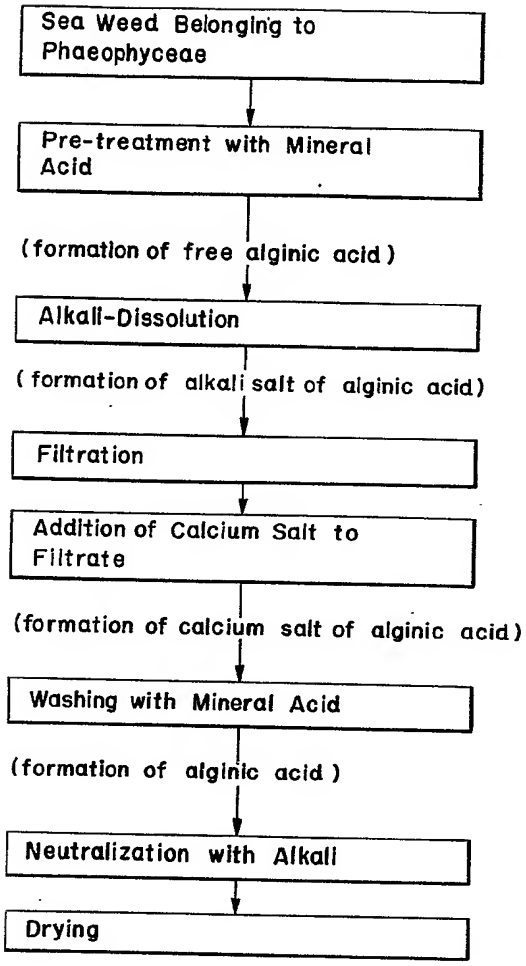
Sea Weed Belonging to Phaeophyceae
Pre-treatment with Mineral Acid
(formation of free alginic acid)
Alkali-Dissolution
(formation of alkali salt of alginic acid)
Filtration
Addition of Calcium Salt to Filtrate
(formation of calcium salt of alginic acid)
Washing with Mineral Acid
(formation of alginic acid)
Neutralization with Alkali
Drying

FIG. 2

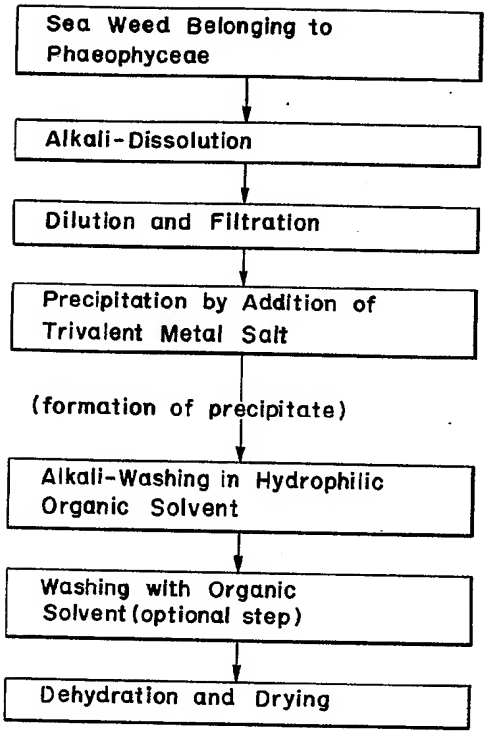
Sea Weed Belonging to Phaeophyceae
Alkali-Dissolution
Dilution and Filtration
Precipitation by Addition of Trivalent Metal Salt
(formation of precipitate)
Alkali-Washing in Hydrophilic Organic Solvent
Washing with Organic Solvent (optional step)
Dehydration and Drying

FIG.3